

official copy
received

8/18/03

DF

#33
attach

Exhibit B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Michael Meyrick Burrell and
Stephen Andrew Coates
Serial No.: 08/192,493 5,830,724
Filed: February 7, 1994
Art Unit: 1804
For: MODIFICATION OF STARCH PRODUCTION
Examiner: Charles C.P. Rories

DECLARATION UNDER RULE 37 CFR 1.132

I, Michael Meyrick BURRELL, of 20 Histon Road,
Cottenham, Cambridge, England, DO SOLEMNLY and SINCERELY
DECLARE as follows:

1. I am the Michael Meyrick Burrell who is named as
being an Applicant in respect of United States
Patent Application Serial No. 08/192,493.
2. I am a scholar of Cambridge University and hold a
PhD in Plant Biochemistry and a Degree in Natural
Sciences with honours in Botany.
3. I initiated and supervised the conduct of the
experiment now to be related.

4. The coding sequence for the wheat homologue of waxy was fused to the high molecular weight glutenin promoter of wheat to produce a chimaeric gene which would be expressed in developing endosperm. A hybrid maize was transformed with this construct using the particle bombardment method and plants were regenerated to produce seed by outcrossing.
5. Analysis of single seeds taken from six independent transformants is shown below. Starch was isolated and the starch granule bound proteins separated by gel electrophoresis. The 60kDa waxy protein is clearly absent from line 3 (Figure 1). The starch from this line clearly has a much reduced amylose content (Figure 2).

Fig.1.

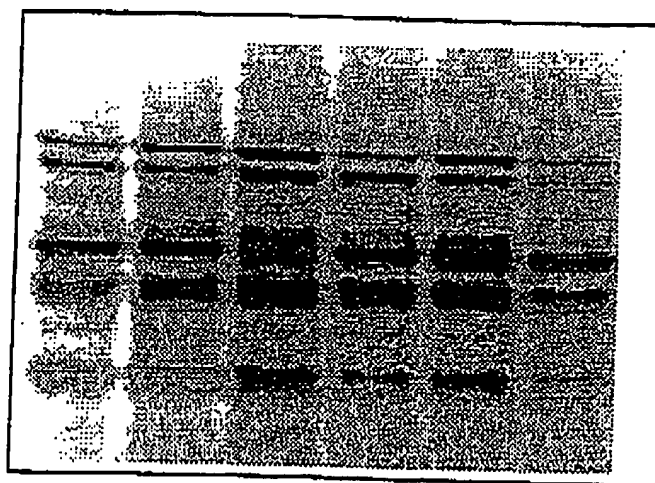
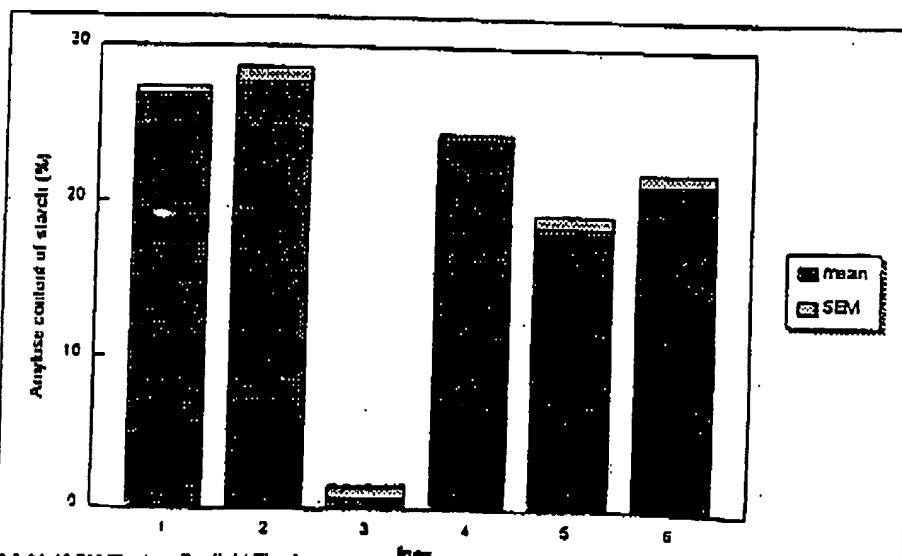


Fig.2.



6. The seed from the progeny of plants obtained in the transformation procedure were divided into two populations. One population contained plants that showed no clear 60kDa waxy protein product and one population contained segregants that had the 60kDa waxy protein. A third population of untransformed controls were also included in the analysis. The results (table below) clearly show the somewhat surprising result that the absence of the waxy protein is associated with no detectable amylose and no change in starch content.

Grain analysed	Starch Content umol/gwt		% amylose		Number of grain analysed
	Mean	S.E.M.	Mean	S.E.M.	
Control line grain	91.30593	13.83537	19.28946	1.834417	5
Progeny grain lacking the product of the waxy gene	105.9155	11.64986	nd*	nd*	15
Progeny grain expressing the product of the waxy gene	92.26848	8.171925	21.92222	0.71156	16

*nd = not detected

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code,

and that such wilful false statements may jeopardise the validity of the application or any patent issuing thereon.

Signature: _____

Michael O. Russell

Date: _____

22nd June 1996